

EFFECT OF STEADY STATE GROWTH RATE PRIOR TO
APPLICATION OF TEMPERATURE SHOCK LOAD ON
THE ABILITY OF A HETEROGENEOUS BIOMASS
TO ACCOMMODATE THE SHOCK

By

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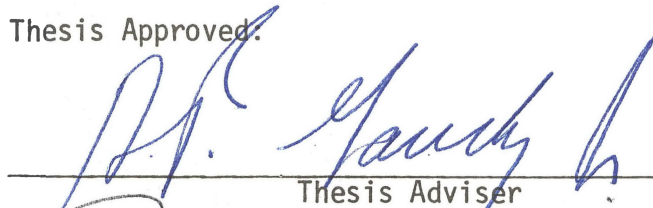
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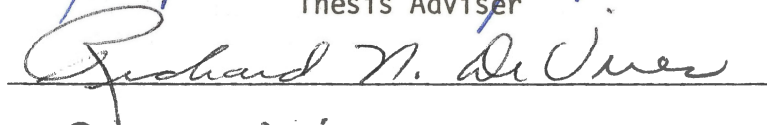
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Dedicated to my wife, Judy

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CHAPTER I

INTRODUCTION

Throughout history man has relied on the flow of streams and rivers to supply water for his various needs and to carry away the waste products of his existence.

It was eventually discovered that a water course carrying the waste of an upstream user became a means of disease transmission. It then became evident that some degree of treatment for a water supply was necessary.

Today it is known that a stream possesses a "self-purification" ability. However, this assimilation capacity of a stream is easily overloaded with organic material. The effect of such overloading is the depletion of the dissolved oxygen supply as the natural course of waste stabilization occurs. The resulting anaerobic conditions produce fish kills, odor problems, and increase the likelihood of disease transmission.

Realizing that a source of water adequate to sustain life was needed, steps were taken to protect the environment. It was decided that industrial and municipal waste effluents must be treated to remove biologically usable organic material.

Treatment of such effluents can be accomplished by either chemical-physical or biological processes. However, biological treatment is more economical and is the most widely used type of treatment.

In order to adequately design and operate a successful treatment plant, a basic understanding of the response of the biomass to environmental conditions is necessary. This study was conducted to gain further insight into the transient biomass response to increases in temperature for cells grown at different specific growth rates prior to the temperature shock.

CHAPTER II

LITERATURE REVIEW

Growth in a system of microorganisms is determined largely by two factors, the properties of the biomass and the environmental conditions to which the biomass is subjected. Since growth is dependent upon a sequence of enzymatic reactions whose rates are temperature related, it would seem that the temperature of the environment would be an important factor to consider.

Bacteria have traditionally been divided into three major categories according to the temperature range which supports growth. In general, these temperature ranges are as follows:

<u>Group</u>	<u>Temperature °C</u>
Thermophiles	40-80
Mesophiles	10-45
Psychrophiles	(-5)-20

The distinction between groups is not as precise as indicated by the temperature ranges shown. For example, some bacteria may be described as psychrophilic due to their ability to grow near 0°C, but may also grow at temperatures characteristic of the upper portion of the mesophilic range. Care should be exercised when the above classification system is used.

Considerable effort has been expended in the study of temperature effects on morphological and physiological characteristics of microorganisms.

Terry, Gaffer, and Sagers (1), using Clostridium acidurici, found

that temperature affects the morphological characteristics of the cells. At 42°C the cells are straight relatively short rods. At 43°C these cells become elongated and at 44°C become filamentous. Dropping the temperature back to 42°C resulted in formation of cross walls and short single cells were formed.

Changes in growth temperature have been studied in relation to changes in cell composition. Evreinova and his co-workers (2)(3)(4)(5) have shown with thermophilic and mesophilic strains of bacteria, fungi, yeasts, and actinomycetes, that as the incubation temperature was increased the RNA and DNA contents decreased, the protein content decreased, and the cell weight decreased. It was also noted that while the DNA composition remained unchanged, the mononucleotide composition of the RNA changed. In other studies by Evreinova and associates (6)(7) using Aspergillus fumigatus and Micromonospora vulgaris respectively, it was shown that thermophilic variants contained decreased amounts of nucleic acids and increased diversity as opposed to the mesophilic variants.

Brown and Rose (8)(9) and McMurrough and Rose (10) have studied the effects of temperature variations of chemostatically grown cultures of Candida utilis. The chemostat was preferred to batch reactors because by hydraulically controlling the growth rate it was possible to separate the metabolic effects caused by changes in temperature from those resulting in changes in growth rate. Their findings showed that the DNA content of cells remained approximately constant while the RNA and protein contents increased as the temperature decreased. The cells were also observed to increase in volume with decreasing temperature; also there was increased synthesis of unsaturated fatty acids.

Tempest and Hunter (11) also observed that decreases in incubation temperature of Aerobacter aerogenes did not cause the DNA content to vary significantly. There were, however, increases in the RNA and carbohydrate contents and a decrease in the protein content as the temperature was decreased. The following hypothesis was formulated to explain their own results and the apparently contradictory results of Schaecter, et al (12) who, using batch cultures, determined that the temperature had no effect on the gross composition of Salmonella typhimurium:

1. The rate of protein synthesis per unit ribosome is constant and independent of growth rate at fixed temperatures and pH values.
2. The concentration of ribosomes within an organism cannot exceed a fixed amount.

Tempest and Hunter further explained that for batch units a lower temperature would decrease ribosomal activity, resulting in decreased protein synthesis reflected in a lower growth rate. If the growth rate is maintained at a constant value, as in a chemostat, then the ribosome content must increase to offset decreased ribosomal activity and to maintain the overall rate of protein synthesis constant. However, if the organisms already maintain their maximum ribosome content, assumed for fast growing organisms in a batch culture, then a decrease in temperature cannot be compensated by further increases in ribosome content. The result is a lower growth rate while maintaining a constant maximum ribosome content and no net change in the RNA content of the cell.

Temperature appears to have an effect on the nutritional requirements of microorganisms. Lichstein and Begue (13) showed that several strains of the mesophilic yeast Saccharomyces, capable of growth on a

glucose-salts medium at 30°C, were unable to grow on the same glucose-salts medium at 38°C. Inoculation into a complex medium of glucose, yeast extract, and casitone did support growth at the higher temperature. It was finally determined that the inability to grow at 38°C resulted from the organism's inability to synthesize pantothenate at the elevated temperature.

The effect of changes of temperature on the growth rate is frequently characterized by the Arrhenius expression:

$$k = Ae^{-\frac{\mu}{RT}}$$

where k is the specific growth rate, A is an entropy constant, μ is the temperature characteristic, R is the gas constant, and T is the absolute temperature.

The temperature characteristic, μ , is a constant characteristic of growth and is equal to the energy of activation, or the number of calories required to activate a mole of the reactant to the energy state required for growth to occur. A plot of the $\log k$ against $1/T$, termed an Arrhenius plot, produces a straight line for the central temperatures of the growth range that rapidly curves and drops to zero at each extreme of the temperature range. The slope of the straight-line portion of the curve is the temperature characteristic, μ .

Ingraham (14) showed that the temperature characteristic of psychrophiles is markedly lower than that of mesophiles.

Ng, et al. (15), using exponentially growing cultures of Escherichia coli, found that abrupt temperature shifts within the temperature range where the temperature characteristic is constant resulted in immediate exponential growth typical of the new temperature.

For shifts made to or from temperatures below the range where μ was constant, a growth rate intermediate to the growth rates of the initial and final temperatures resulted. The conclusion was that growth at low temperatures alters or damages the cell in a way that reduces growth rate.

Shaw (16) performed similar experiments with mesophilic and psychrophilic yeasts. The results agreed with those of Ng, et al., (15). Shaw also showed that shifts to and from high temperatures resulted in transient growth rates. The results also indicated that the transient growth rates followed the Arrhenius function with modifications allowed for cell damage at the new temperature.

After publication of the work of Graham-Smith (17), it was generally accepted that maximal cell counts occur at a temperature considerably below the temperature of most rapid growth. However, Sinclair and Stokes (18), believing this phenomenon is due to increased amounts of dissolved oxygen at low temperatures, showed that vigorously aerated cultures produced the same yield (defined as maximal cell count) at 30°C as they did at 10°C, i.e., when oxygen was not the limiting factor.

Using the yeast strain Saccharomyces cerevisiae, Jones and Hough (19) demonstrated by shaker studies at 25°C and 38°C that the elevated temperature had the effect of increasing the maximum growth rate, μ_{\max} , and saturation constant, K_s , and decreasing the cell yield constant, Y . Studies were also conducted using a glucose-limited medium in chemostats. Although there was a reported decrease in the weight of cells per milliliter of medium (termed yield by the authors) it is difficult to apply these results because there is some doubt that the system is indeed fully aerobic.

It is important to point out the confusion that occurs in discussion of cell yield in the literature. The most frequently used definitions are maximal cell count and dry mass of organisms produced per mass of substrate utilized. Hereafter, all references to yield pertain to the latter definition.

Batch studies by Senez (20) with Aerobacter aerogenes indicated a constant yield in the range of 22°C to 37°C. Above 37°C, the yield declined sharply until it reached a value of zero at 42°C. In the same temperature range there was a corresponding drop in the growth rate. On the basis of the data obtained, it was hypothesized that biosynthetic reactions do not maintain a balance with catabolic reactions at high temperatures, a situation termed energy uncoupling.

Similar studies by Ng (21) on Escherichia coli under conditions of decreasing temperatures produced results of the same nature. The cell yield appeared to be independent of growth rate until rates were attained falling below those which coincide with those at which the growth rate deviates from the straight-line portion of the Arrhenius plot.

Returning to the work of Brown and Rose (8) using a glucose-limited chemostat, the yield tended to increase as the temperature decreased from 30°C to 20°C at various fixed dilution rates of .20, .10, and .05 hours⁻¹.

A statistical analysis on the growth of Saccharomyce cerevisiae performed by Eroshin, et al. (22) indicated that the cell yield was a function of two independent variables, pH and temperature. This work was carried out using a glucose-limited media fed at a rate of 0.1 hr⁻¹. The results were presented in a form similar to a topography map with

the temperature as the abscissa, pH as the ordinate, and the contour lines representing the yields. The results indicated the maximum yield occurred when $\text{pH} = 4.1$ and $T = 28.3^{\circ}\text{C}$.

It has been suggested that the properties of water, such as density, specific heat, viscosity, dielectric constant, index of refraction, and others, undergo abrupt transitions at various temperatures. These temperature anomalies, or "kinks", are a result of molecular structure changes in the liquid phase, as suggested by Drost-Hansen (23). The kinks are observed most frequently at approximate temperatures of 15°C , 30°C , 45°C , and 60°C and occur within a range of $\pm 2^{\circ}\text{C}$. That is to say, the 15°C kink would occur between 13°C and 17°C .

It had been postulated earlier by Drost-Hansen (24) that the kinks affected biological activity. To test this hypothesis, Oppenheimer and Drost-Hansen (25), performing batch studies on an unnamed sulfate-reducing bacterium, correlated growth with the temperatures of the predicted kinks. Multiple optima were observed at 11°C , 25°C , and 39°C , i.e., temperatures approximately in the middle of two consecutive kinks. Multiple minima occurred at 16°C , 31°C , and 43°C , the approximate temperatures of the kinks.

In order to confirm the above findings, Davey, et al., (26) used four bacteria to cover the temperature range from 5°C to 70°C : Pseudomonas fragi (5°C to 25°C), Streptococcus faecalis (20°C to 40°C), Bacillus coagulans (35°C to 55°C), and B. stearothermophilus 1518 smooth (50°C to 70°C). In all cases a repression of growth occurred within $\pm 2^{\circ}\text{C}$ of 15°C , 30°C , 45°C , and 60°C .

Up to this point, all the studies of continuous cultures cited were carried out under steady-state conditions. The study of the transient

response to changes in temperature is also necessary to gain a better understanding of the dynamic behavior of continuous cultures.

Ryu and Mateles (27) studied the transient response of Escherichia coli B to step increases and decreases in temperature. The transient response was determined by monitoring the nitrogen-limited medium (NH_3 was the source of nitrogen) and calculating a transient growth rate. The responses to four temperature changes were studied. In two cases the temperature was shifted from 37°C to 32°C and 37°C to 27°C . The ammonia concentration in the effluent increased from its initial steady-state value at 37°C to a new steady-state value at the lower temperature. The effect on growth rate was an immediate decrease to its transient minimum with a gradual return to a growth rate equal to the dilution rate. The cultures appeared to give a better response for the 5°C downshift than the 10°C downshift, i.e., smaller difference between the transient minima and the steady-state growth rates. It is important to note that the comparison of responses would be more easily justified had the dilution rate been the same in each case.

The other two temperature shifts studied were increases from 32°C to 37°C and 27°C to 37°C . In each case the ammonia concentration decreased to a new steady-state level. The effect on growth rate was an immediate increase to a transient maximum and a gradual decrease back to its steady-state level. The difference between the transient maximum values and steady-state values was greater for the 10°C increase than the 5°C increase. Again analysis of the results would have been more readily effected had the dilution rates been equal. It is noteworthy that the transient maxima or minima were less than the growth rates predicted by the Arrhenius equation.

Topiwala and Sinclair (28), investigating both steady-state and transient behavior of continuously cultured Aerobacter aerogenes, found that the true yield appeared to be independent of temperature, but the observed yield, Y_0 , (i.e., the μ dependent value of cell yield) was maximum around 25°C while the maximum growth rate was highest around 40°C. The temperature dependence of the maximum growth rate, saturation constant, and endogenous metabolism constant, k_d , was plotted in Arrhenius form.

Step up temperature changes from 25°C to 35°C and step down changes from 35°C to 25°C were performed to assess the transient responses. The step up change produced an immediate decrease in the substrate concentration in the reactor along with an increase in solids concentration. The step down change had the reverse result. The substrate concentration increased and the solids concentration decreased.

The discussion thus far presented has pertained to the effect of temperature on pure cultures. As the waste treatment process does not consist of a pure culture, it is important to study the relationship of temperature on heterogeneous or natural populations. One of the earliest attempts to study the effects of temperature in the waste treatment field was reported by Bloodgood (29). Studies of an activated sludge plant in Indianapolis led to the conclusion that an increase in temperature from 10°C to 30°C uniformly increased the oxygen utilization rate. Further studies by Bloodgood (30) at the Indianapolis treatment plant showed a definite relationship between aerator detention time and temperature, i.e., the detention time required to maintain satisfactory removal decreased as the temperature increased.

Sawyer and Nichols (31), studying the effect of temperature and

sludge concentration on oxygen utilization, found that the rate of consumption is much greater at higher temperatures. Studies by Sawyer and Rohlich (32) indicated that low winter temperatures were conducive to the development of non-nitrifying sludges and summer conditions to the development of nitrifying sludges.

Other experiments conducted by Sawyer (33) showed that equivalent BOD removals were obtained under all conditions except low solids concentrations at 10°C. This was in direct opposition to the aforementioned studies of Bloodgood (30). These studies also confirmed the studies by Sawyer and Rohlich (31) that the proportion of nitrifying bacteria decreased at low temperatures.

The effect of temperature on biological growth rates in wastewater treatment systems has been characterized by a modified Van't Hoff-Arrhenius equation:

$$k_T = k_{20} \Theta^{(T - 20)}$$

where k_T is the growth rate at temperature T , k_{20} is the growth rate at 20°C, T is the temperature, °C, and Θ is the temperature coefficient. This temperature coefficient, Θ , was usually assumed to have a value of 1.047 within the range of 2°C to 40°C. However, Gottas (34), as a result of a statistical analysis, concluded that three different values were necessary to describe the rate changes:

<u>Temperature Range</u>	<u>Θ</u>
5°C - 15°C	1.100
15°C - 30°C	1.041
30°C - 40°C	0.9657

Another result obtained in this study of the effects of temperature on biochemical oxidation was that the lag period increased as the

temperature decreased. It was also determined that considerable nitrification occurred at temperatures of 30°C and greater.

Dougherty and McNary (35) found in studies on the treatment of citrus wastes that an activated sludge unit could be operated at temperatures as high as 36°C with no deleterious effects to the degree of treatment. A rapid rise in temperature of 4°C or 5°C in approaching the 36°C temperature temporarily disrupted the system. However, the system recovered if no further increase took place. The number and activity of the protozoa decreased as the temperature increased from 36°C to 43°C. The treatment was poor at 46°C and the experiment was concluded.

In studies by Ludzack, et al. (36), it was found that two weeks were required for a population to approach equilibrium after a significant change in temperature. They also noted that flocculation was inferior at low temperatures and there was a substantial increase in solids production at the low temperatures.

Hurwitz, et al. (37), in response to a problem at a Chicago sewage treatment plant, studied the effect of temperature on cellulose degradation by activated sludge. It was determined that the activated sludge process was extremely sensitive to low temperatures as far as cellulose stabilization was concerned.

Operating trickling filters at 18°C and 55°C, Husmann and Malz (38) determined that the degree of purification of a phenol waste was essentially the same at both temperatures. However, the ammonia content of the effluent from the thermophilic trickling filter was approximately twice that of the mesophilic trickling filter. Activated sludge experiments were performed ranging from 20°C to 55°C. Optimal activity

occurred at 30°C with greater ammonia concentrations being present in the effluent at higher temperatures. It appeared that nitrification was impeded at the higher temperatures.

Keefer (39), analyzing the operational results of an activated sludge plant over a period of 20 years, concluded that a temperature increase from approximately 12°C to about 24°C resulted in higher percentages of BOD removal and suspended solids removal.

Carpenter, et al. (40), studying the treatment of a variety of pulp and paper mill wastes, determined that the optimal treatment occurred at 37°C and decreased rapidly above 37°C. Adverse effects by temperature reductions below 20°C were compensated by increases in retention time.

Investigation of ATP response of activated sludge at various temperatures by Brezonik and Patterson (41) led to the conclusion that abrupt temperature changes up to 37°C had a negligible effect on the biomass. As no actual temperature shift experiments were performed, this conclusion was unsubstantiated.

Simultaneous operation of three activated sludge units with recycle by Benedict and Carlson (42) indicated that acclimation was more rapid to low temperature ranges (4°C) than to higher temperatures (30°C) with initial seed from a 19°C culture. After acclimation at 4°C and 32°C, a return of both systems to 19°C evoked a better response from the system which had formerly been operated at 4°C.

Operating a bench scale model with internal recycle, Friedman and Schroeder (43) concluded that the Arrhenius equation was not acceptable for design of activated sludge. The variation of θ , the temperature coefficient, over the range of 37°C to 47°C was attributed to increasing

competition between mesophilic and thermophilic microorganisms. It was also the authors' opinions that both the maximum specific growth rate and yield was greatest at 20°C although the values of the cells yield are somewhat questionable since they recorded some yield values greater than unity.

Muck and Grady (44) operated a once-through reactor with a heterogeneous population to observe the effect of temperature on the measured values of solids and effluent substrate. From their data, it was determined that the maximum growth rate and decay rate coefficient increased with increase in temperature, i.e., an Arrhenius function, in the 10°C to 30°C range. The decay coefficient exerted a greater influence at longer cell residence times, as expected. The true yield was observed to increase with increases in temperature from 10°C to 20°C but fell with further increases in temperature. The effect of increased temperature on the saturation constant was inconclusive but a general increase was indicated.

A comment by Randall (45) concerning the above work indicates that ignoring a point at 20°C in the decay rate values was not justified and he showed other examples of mixed populations that gave variable decay rate coefficients.

Concerned with the production of single cell protein, Surucu, et al. (46), developed arguments for the thermophilic treatment of certain industrial wastes. The values of the maximum specific substrate utilization rate, μ , the saturation constant, k_s , and the decay coefficient, k_d , were higher in the thermophilic range than the mesophilic range although the true yield, Y_t , was lower, similar to the results of Muck and Grady (44).

Studies by George (47), George and Gaudy (48), and Gaudy (49) at the Bioenvironmental Engineering Laboratories at Oklahoma State University were performed to investigate the effect of subjecting heterogeneous populations to increases and decreases in temperature from a baseline steady-state temperature of 25°C . Responses of systems with dilution rates of 0.25 hr^{-1} and 0.125 hr^{-1} were analyzed on the basis of identical temperature changes.

A temperature shift of 25°C to 8°C indicated the effect of the temperature shock was so great at either specific growth rate that neither of the simultaneously shocked systems ever recovered. However, a milder shock of 25°C to 17.5°C gave evidence that the slower growing system, i.e., $D = 0.125\text{ hr}^{-1}$, responded more favorably as indicated by less substrate leakage.

A typical pattern of responses to increases in temperature involved a dilute-out of biological solids and an increase in effluent COD upon initiation of the change followed by recovery of the system. A change in predominance was evident in these temperature upshifts which may account for variations in the composition of the biomass. As in the temperature downshift experiments, there was little doubt that the slower growing systems responded more favorably to the temperature changes.

Comparison of all the temperature shifts indicated that a system initially acclimated to a 25°C environment accommodated upshifts easier than downshifts of temperature. Except for the extreme temperature downshift which completely disrupted the systems, the cell yield had a tendency to become decreased at higher temperatures compared to the preshock steady state.

CHAPTER III

MATERIALS AND METHODS

General Experimental Plan

The general experimental plan was to determine if the immediate past growth history affected the response to temperature shock loadings. Accordingly two related kinds of experiments were undertaken. In one experiment, heterogeneous microbial populations were grown in a completely mixed reactor (a chemostat) at a dilution rate, $D = 0.125 \text{ hr}^{-1}$. After assessing the steady state behavior of the system the mixed liquor was equally proportioned to two small chemostats and the dilution rate was set at 0.125 hr^{-1} and 0.25 hr^{-1} . At the same time a temperature shock (increase) was administered at the same rate in both systems. The effects on effluent and biomass were then assessed. The second type of experiment was essentially the same as that described above except that the cells were grown at $D = 0.25 \text{ hrs}^{-1}$ prior to the increase in temperature.

Composition of Synthetic Waste

The synthetic waste was designed in such a manner as to make the carbon source, glucose, the limiting nutrient. Concentrations of all constituents in the growth medium are given in Table I.

TABLE I
COMPOSITION OF SYNTHETIC WASTE

Glucose	1000 mg/l
$(\text{NH}_4)_2\text{SO}_4$	500 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100 mg/l
MnSO_4	10 mg/l
CaCl_2	7.5 mg/l
FeCl_3	0.5 mg/l
KH_2PO_4	581.25 mg/l
K_2HPO_4	1867.5 mg/l
Tap Water	100 ml/l
Distilled Water	To Volume

Source of Microbial Population

All experiments were started using an initial seed of microorganisms obtained from the effluent of the primary clarifier of the municipal treatment plant in Stillwater, Oklahoma.

Development of Cells at the Desired

Specific Growth Rate

The seed organisms were inoculated into 5 liters of synthetic waste and allowed to grow up in a batch operation for 1 to 2 days. Following this the feed was pumped continuously (Milton Roy MM2-B-96R) at unit flow rates, D , of either 0.125 hr^{-1} or 0.25 hr^{-1} . The reactor was checked for complete mixing frequently and the system was adjudged to be in a steady state condition when biological solids, X , and effluent COD, S , obtained unchanging concentrations. The reactor was immersed in a water bath and the temperature of the mixed liquor was maintained constant at 25°C . The behavior of the system under steady state growth was assessed by measuring the biological solids concentration, X , using the membrane filter technique (HA, $0.45\mu\text{m}$ pore size) (50). The filtrate was analyzed for chemical oxygen demand (COD) (50) and for its carbohydrate content using the anthrone test (51). The medium was well buffered at pH 7 but periodic checks on pH were made. After defining the steady state condition shock loading experiments were conducted as described below.

Shock Loading Studies

Shock loading studies were initiated by transferring equal portions of the aerating mixed liquor from the five liter reactor to each of two

smaller chemostats of 2.3 liter capacity previously described by George (47). One of these chemostats was set at the same dilution rate as that of the five liter reactor (either 0.125 or 0.25 hrs^{-1}) and the other was set at either $.125$ or 0.25 hrs^{-1} . For example, if the cells had been previously growing at $D = 0.125 \text{ hrs}^{-1}$ there was, after parting the system, two systems, one growing at 0.125 hrs^{-1} and the other at a dilution rate of 0.25 hrs^{-1} . Simultaneously with this reportioning of the system the temperature setting in the water bath (Precision "Lo/Temptrol") was increased from 25°C to 36°C . Frequent samples were taken for assessing biological solids, COD, and carbohydrate. Also the temperature in each reactor was measured frequently in order to determine the rate at which the new water bath temperature was attained.

Both types of experiments were run a number of times. The intent was to assess the behavior in the immediate transient in response to the step change in temperature and it was also intended to determine if a new steady state would eventually be obtained.

The tests supplied for complete mixing of the reactors were those previously employed in the author's laboratory, i.e., adherence to the theoretical dilute in or dilute out patterns for soluble substrate and equal optical density in the reactor and the reactor effluent.

CHAPTER IV

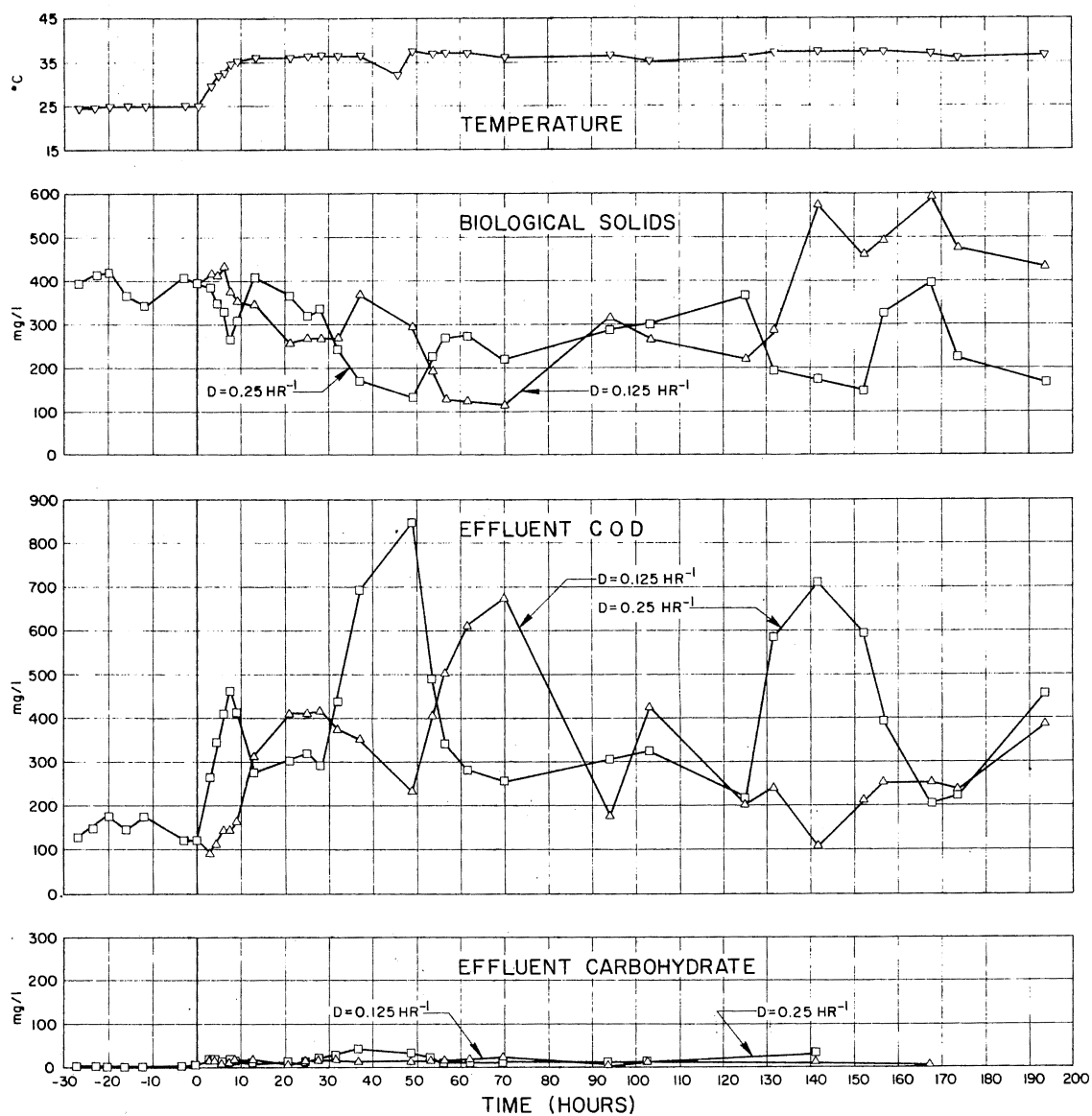
RESULTS

Past Growth History of $D = 0.25 \text{ hr}^{-1}$

Figure 1 shows the shock load results for a system initially growing at a dilution rate of $D = 0.25 \text{ hr}^{-1}$ at a base temperature of 25°C . At time zero the unit was proportioned into two smaller units, one with a dilution rate of $D = 0.25 \text{ hr}^{-1}$ and the other with a dilution rate of $D = 0.125 \text{ hr}^{-1}$. The temperature was increased in both systems to 36°C . The unit with the higher dilution rate experienced an immediate decrease in biological solids. There was a corresponding rise in the effluent COD. It reached a concentration of 471 mg/l seven and one-half hours after the temperature shock was initiated. The slower growing unit, $D = 0.125 \text{ hr}^{-1}$, showed an immediate rise in the solids level followed by a dilute-out of the cells. The effluent COD reached a plateau approximately eleven hours after the shock was initiated.

Both units made partial recoveries as indicated by the decline of the effluent COD concentration. However, a second loss of cells occurred. For the fastest growing unit, substrate leakage reached a maximum of 849 mg/l forty-nine hours after application of the shock load, while the slower growing unit reached a peak of 677 mg/l after seventy hours of operation at the elevated temperature. The effect was less severe and slower to occur for the slower growing unit. The carbohydrate content in the filtered effluent remained relatively low,

Figure 1. Response of a System With an Initial Specific Growth Rate of $D = 0.25 \text{ hr}^{-1}$ to an Increase in Temperature at the Time of Proportionment into Two Units, One With a Dilution Rate of $D = 0.25 \text{ hr}^{-1}$ and One With a Dilution Rate of $D = 0.125 \text{ hr}^{-1}$

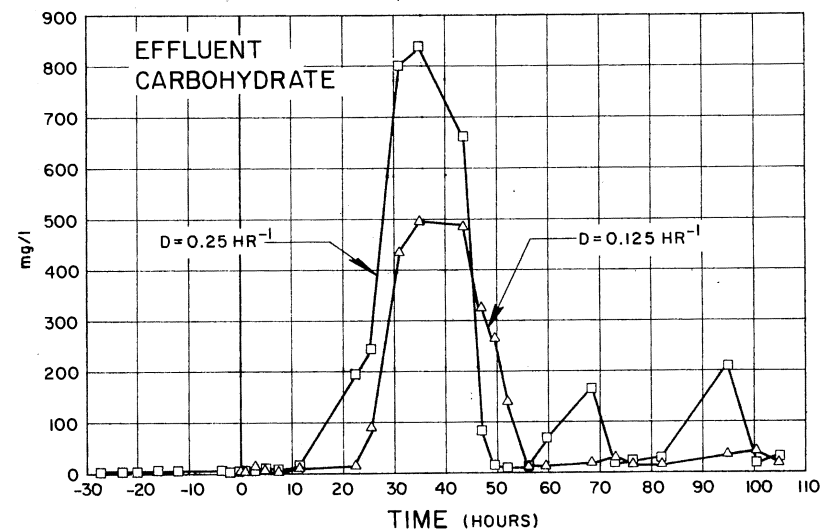
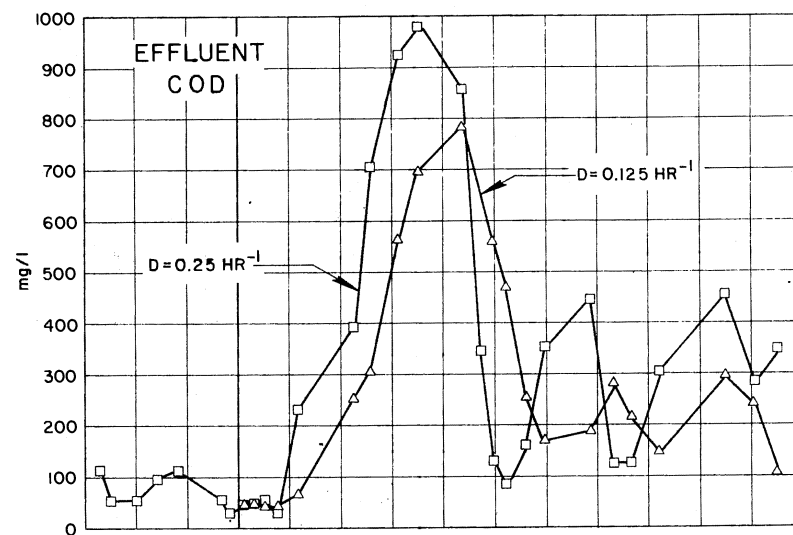
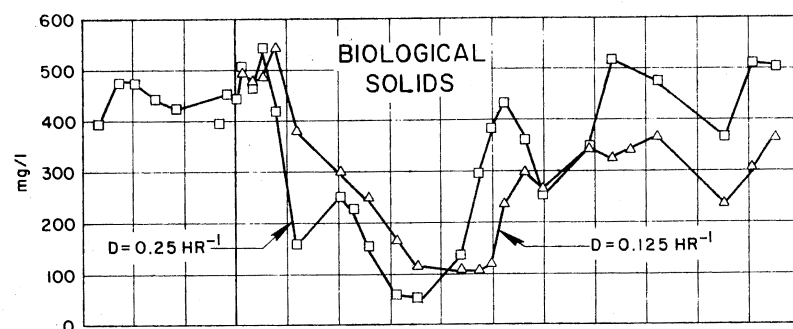
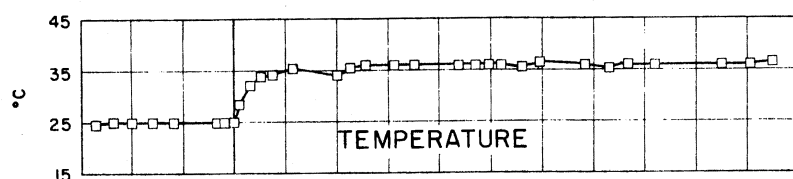


less than 50 mg/l, indicating that the high COD values resulted from metabolic intermediate or end products.

Flocculation of the cells was noted in both units, occurring at 131½ hours for the slower growing unit and at 152 hours for the unit with the highest dilution rate. Neither unit had achieved a steady state condition comparable to the preshock state by the time of terminating the experiment.

Figure 2 shows a second experimental run under the same conditions. After initiation of the temperature shock load, the solids level of both units fluctuated for the first seven hours, after which a rapid decline occurred. A minimum concentration of 52 mg/l of biological solids was reached 35 hours after the temperature was increased in the unit with a dilution rate of $D = 0.25 \text{ hr}^{-1}$, while the effect was not quite as severe in the slower growing unit. As expected with such low levels of biological solids, a rise in the effluent COD was observed. Maximum leakage occurred in the faster growing unit, 979 mg/l. The unit with a dilution rate of $D = 0.125 \text{ hr}^{-1}$ reached a peak of 782 mg/l. As indicated by the anthrone test for carbohydrate analysis, the leakage was a result of large amounts of carbohydrates, possibly the original substrate and not end products or intermediates as was the case of Figure 1. A definite change of predominance occurred in both units after 52 hours of operation at the elevated temperature as observed by a change of color of the mixed liquor from white to green. Again a final steady state was not observed. Both units became flocculant after 95 hours of operation at the elevated temperature.

Figure 2. Response of a Second System With an Initial Specific Growth Rate of $D = 0.25 \text{ hr}^{-1}$ to an Increase in Temperature at the Time of Proportionment into Two Units, One With a Dilution Rate of $D = 0.25 \text{ hr}^{-1}$ and One With a Dilution Rate of $D = 0.125 \text{ hr}^{-1}$



Past Growth History of $D = 0.125 \text{ hr}^{-1}$

Figure 3 shows the steady state development and response to temperature shock load for a system initially growing at a dilution rate of $D = 0.125 \text{ hr}^{-1}$. At time zero system was then divided into two units with dilution rates of $D = 0.125 \text{ hr}^{-1}$ and $D = 0.25 \text{ hr}^{-1}$. The biological solids began an immediate washout in both units. The two units reached a minimum point in solids concentration at approximately the same time. However, the unit with the lower dilution rate, $D = 0.125 \text{ hr}^{-1}$, was not affected quite as severely as the other unit. While the biological solids difference was only 100 mg/l, the difference in substrate leakage was quite substantial. The maximum leakage was 678 mg/l for the unit with the greater dilution rate and 278 mg/l for the unit with the lower dilution rate. A corresponding increase in the effluent carbohydrate content is also noticeable. At the higher growth rate the leakage contained a significant amount of carbohydrate, approximately two-thirds. At the lower dilution rate only one-third of the effluent COD was composed of carbohydrates.

The solids level contained to fluctuate for the duration of the experiment. The effluent COD varied somewhat until $137\frac{1}{2}$ hours after the shock was initiated when it became quite steady in both units. The effect on the carbohydrate content was the early rise in both units and a very steady content, less than 20 mg/l, thereafter.

Preshock development generated a biomass with a white color. After operation at the elevated temperature for $113\frac{1}{2}$ hours, the unit with the lower dilution rate experienced a change in color. A light brown color developed at this time. Six hours later the unit turned dark green and remained that color until termination of the project.

Figure 3. Response of a System With an Initial Specific Growth Rate of $D = 0.125 \text{ hr}^{-1}$ to an Increase in Temperature at the Time of Proportionment into Two Units, One With a Dilution Rate of $D = 0.25 \text{ hr}^{-1}$ and One With a Dilution Rate of $D = 0.125 \text{ hr}^{-1}$

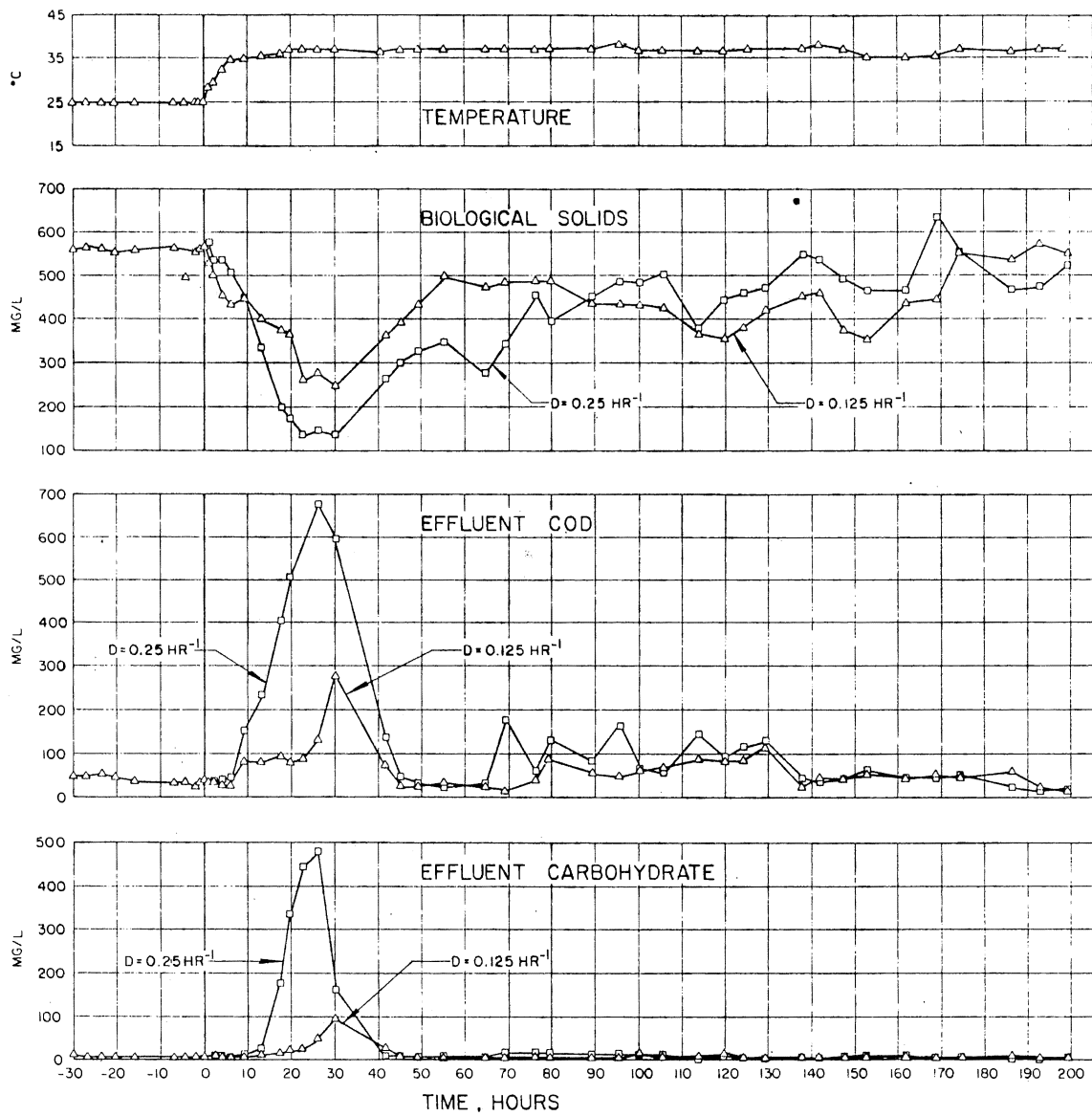


Figure 4. Response of a Second System With an Initial Specific Growth Rate of $D = 0.125 \text{ hr}^{-1}$ to an Increase in Temperature at the Time of Proportionment into Two Units, One With a Dilution Rate of $D = 0.25 \text{ hr}^{-1}$ and One With a Dilution Rate of $D = 0.125 \text{ hr}^{-1}$

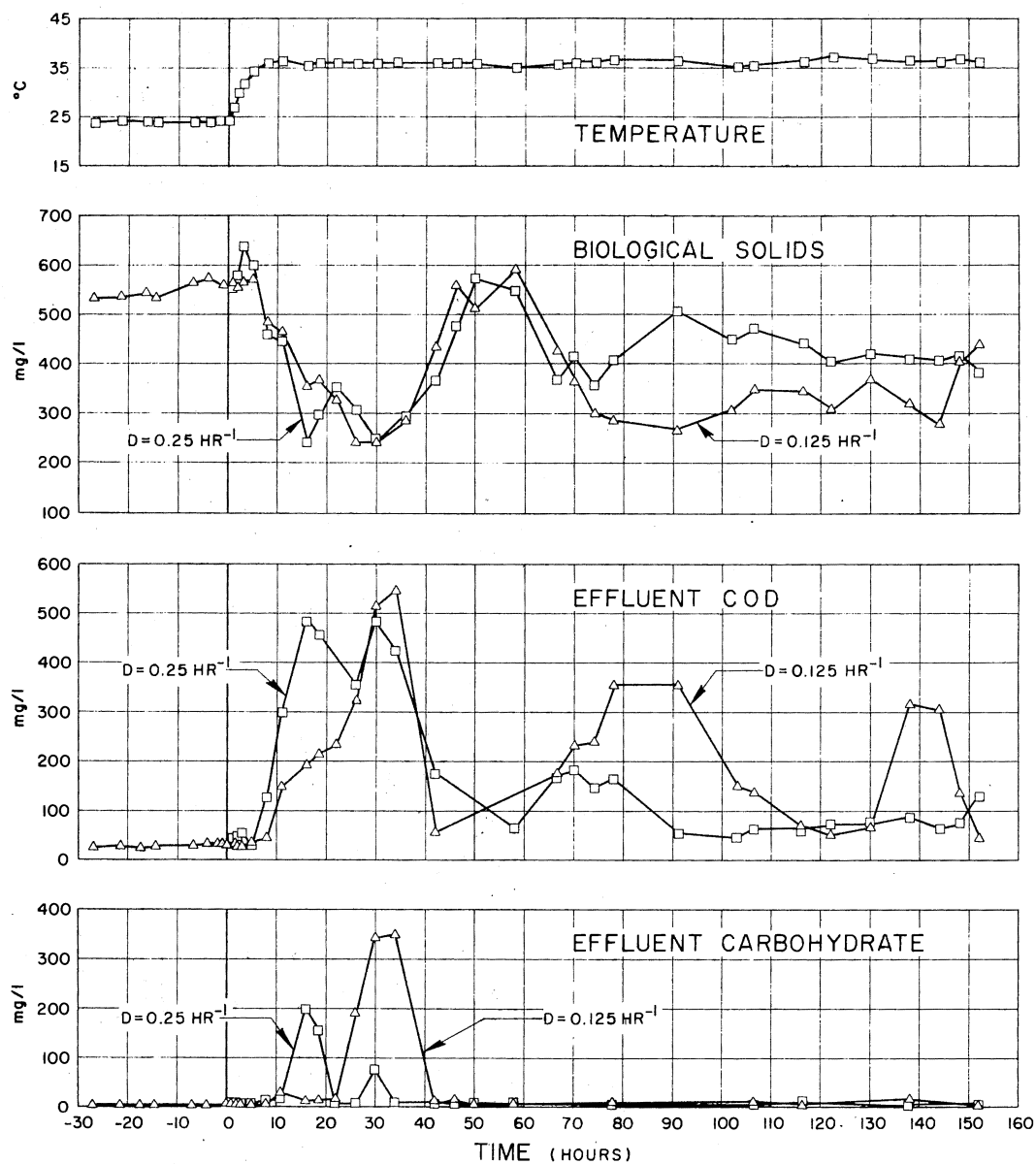


Figure 4 depicts another run of the type shown in Figure 3. Although there was a slight initial solids buildup in the first five hours, dilute-out soon followed. The chemostat with the highest unit flow rate lost solids more rapidly. After the appearance of what seemed to be the initiation of a recovery, a second loss of solids occurred, possibly due to a predominance change. The effluent COD reflects the trends shown by the solids. The minima for both units is approximately 240 mg/l of biological solids thirty hours after the shock was initiated. Both recovered at approximately the same rate. About sixty hours of operation at the elevated temperature produced a second dilute-out of cells. Both units reached relatively stable steady states with the unit at the higher dilution rate attaining the higher biological solids level.

This was the only experiment performed in which the slower growing organisms, i.e., $D = 0.125 \text{ hr}^{-1}$, has a greater substrate leakage although the difference between the maxima is relatively insignificant. However, as indicated by the plot of carbohydrate content in the effluents, the unit with the faster dilution rate actually utilized a greater amount of the carbohydrate. After 40 hours of operation, nearly all the glucose fed was utilized in both systems. Thus, the leakage of COD was due to release of metabolic intermediate products.

CHAPTER V

DISCUSSION

It is recalled that the purpose of this investigation was to determine whether the immediate past growth history had an effect on the response of heterogeneous populations to increases in temperature. Previous work by George (47) had indicated that growth at lower specific growth rates allowed better accommodation to several types of shock loads, including temperature shock loads. However, his experiments allowed the development of different populations in each reactor prior to the shock. The experiments in this study were designed in such a manner that the same microbial population was initially present in each reactor and the differences in response could be attributed directly to the differences in dilution rates at the time the shock load was applied. For each particular run, both the change in temperature and the rate of administering the temperature shock loads were the same for both systems.

Figures 1 and 2 showed the response of a heterogeneous population initially growing at a dilution rate of $D = 0.25 \text{ hr}^{-1}$ subjected to an increase in temperature. In Figure 1 leakage of large amounts of organic material occurred but the carbohydrate content of the filtered effluent was quite low and steady. The leakage was not due, therefore, to sudden inactivity of the biomass. Rather the high concentration of oxygen demanding material resulted from intermediate organic products

formed during the metabolism of glucose. Such accumulation of metabolic products might be more indicative of very rapid growth rather than retarded growth. The build up and eventual decline of the effluent COD levels may have resulted from selection of a new metabolic pathway under the influence of the increased temperature as suggested by Oppenheimer and Drost-Hansen (25). The decline may also have been a result of a shift in predominance although no gross change was apparent.

With respect to leakage of carbohydrate, the results shown in Figure 3 are quite different than those shown in Figure 1. Similar patterns of cell dilute-out and COD leakage occurred, but in the case of Figure 2 the high COD content of the effluent was almost entirely due to the presence of carbohydrates. In all probability, this high carbohydrate content is the original glucose substrate and is indicative of the system's failure to effectively degrade the original substrate. As a definite predominance change was observed after 52 hours of operation, it may be suggested that the initial population in each unit was unable to respond to the new environment and diluted out of the system. The period of high leakage and eventual decline could indicate the rise of new predominating species. Although there were some differences in response, it can be said that the responses were in the main similar; the system which was growing more slowly leaked less substrate COD.

Figures 3 and 4 showed results for systems growing at an initial dilution rate of $D = 0.125 \text{ hr}^{-1}$. In Figure 3, as the shock load was applied, both systems departed from the steady state and exhibited a major disruption followed by a recovery and return to relatively steady conditions. The system with the lower dilution rate responded much more favorably than the faster growing system. The unit growing with

$D = 0.25 \text{ hr}^{-1}$ may have experienced a predominance change between hours 64 and 129 which may account for the slight fluctuation in effluent COD during this time, although no visible signs, such as the color change of the mixed liquor which occurred in the slower growing system, were noted. Figure 4 showed the response to a similar shock condition. The results were somewhat different from those of Figure 3 but in the main the total amount of COD in the filtrate was higher for the faster growing system during the first disruption-recovery period. That is to say, the area under the COD curves during the first 42 hours was greater for the faster growing biomass.

In all systems there was an initial response consisting of substrate leakage and recovery followed by a secondary or after response which led to fluctuations in biological solids and COD. The secondary response led to considerable loss of substrate but did not appear to be correlated to specific growth rate. The maximum concentration of COD leaked was slightly higher for the two systems operated at the faster growth rate, μ , before applying the shock. However, the general trend in regard to initial response was that regardless of the growth rate prior to the shock, the system growing at the lower dilution rate at the time of the shock responded better, i.e., leaked less oxygen demanding material, than the system with the higher unit flow rate.

CHAPTER VI

CONCLUSIONS

1. For the two specific growth rates herein employed, the previous growth history has little influence on the response to an increase in temperature from 25°C to 36°C.
2. The system with the lower dilution rate at the time the shock load is applied gives a better response to the shock load, i.e., there is less leakage of soluble organic material.
3. The increase in temperature may cause shifts in the predominating species in systems employing heterogeneous populations. These shifts may cause secondary disruptions of the system's effluent characteristics.

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